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HRV and EEG based Indicators of Stress in Children with Asperger Syndrome in Audio-visual Stimulus Test

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***Abstract*—Asperger syndrome (AS) is a neurobiological condition which is characterized by poor skills in social communication, and restricted and repetitive patterns of behavior and interests. We studied whether stress-related indices of heart rate variability (HRV) and electroencephalography (EEG) are different in children with AS than normal controls. We analyzed retrospectively the data of the test where audiovisual stimuli were used. We hypothesized that this test is a stressful situation for individuals with AS and they would have a greater reaction than control subjects. EEG and one-channel electrocardiography (ECG) were collected for children with diagnosis of AS (N = 20) and their age-matched controls (N = 21). HRV indices, frontal EEG asymmetry index and brain load index were calculated. HRV based indices revealed increased sympathetic activity during the test in children with AS. EEG based indices increased more in children with AS during the test compared to baseline. Thus, the children with AS seems to have a greater reaction to stressful situation.**

I. INTRODUCTION

STRESS means changes in organism as respond to mental, emotional or physical demands. Even though stress has an unpleasant tune all the stress is not necessarily bad but in certain situations stress can improve performance. In a stressful situation both cellular and systemic level processes are activating. One of the important mechanisms in the stress is the changes in hypothalamus, hypophysis and adrenal gland -level which is responsible to segregate adrenocorticotrophic hormone (ACTH) [1]. ACTH is known as a stress hormone which stimulates sympathetic nervous system [2]. In heart rate variability (HRV) the decreased level of high frequency (HF, 0.15-0.4Hz) power and increased level of low frequency (LF, 0.04-0.15Hz) or LF/HF relations are observed during the stress tests [3]. HF is usually connected to activation of parasympathetic branch of autonomic nervous system while LF is mostly connected to sympathetic branch [4]. In addition heart rates have been reported to increase during stress [4].

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Stress and different emotions also affect EEG. Prefrontal cortex has an important role in brainwork and emotion and observation control. It has been observed that positive emotions increases alpha activity in left prefrontal cortex while negative emotions increase alpha activity in right side [6, 7]. Activity difference between right and left prefrontal cortex is called asymmetry [6]. Stress is connected to increased activity in right prefrontal cortex. [7]. From EEG studies it is known that increased cognitive brain load increases frontal theta activity and decreases parietal alpha activity [8]. Based on this, Holm et al recently proposed a novel brain load index which reacts to both acute external and cumulative internal load [9].

Asperger syndrome (AS), an autism spectrum disorder (ASD), is a neurobiological condition persisting throughout childhood up to adolescence. Prevalence is 0.36-0.48% in the school age population. The AS is characterized by poor skills in social communication, restricted and repetitive patterns of behavior and interests [10,11]. Their cognitive and linguistic development does not have delay like generally in autism spectrum disorders. Individuals with AS have difficulties to recognize and express emotions [12]. They may have problems to manage stress [13]. In addition higher levels of plasma ACTH have been found from adults with AS indicating chronic stress [14]. Scannings and neuropsychological tests have revealed some abnormalities in the brain of the individuals with AS, mostly in frontal and temporal lobe [10].

Simultaneous stress related changes in HRV and EEG have been found in sleep studies of normal subjects [15]. This paper aims to study simultaneous stress related changes in HRV and EEG on children with AS. We hypothesized, that individuals with AS would have greater reaction to stressful situation than control subjects. We selected EEG and HRV indices from the literature that have been used to evaluate the stress and emotions and aimed to study the differences between persons with AS and healthy controls during the stress related test.

II. METHODS

A. Participants

Data was obtained from 20 children with diagnosis of AS (N = 20, age 10.7±0.9 years, 14 boys) who were recruited through the Clinic of Child Psychiatry, Oulu University Hospital. Their IQs were tested to be in normal range. The control group consisted of 21 typically developed, age-matched children who were volunteers from local schools (N

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= 21, age: 10.9±1 years, 12 boys). All control children came from comparable socio-economic backgrounds, and they were native Finnish speakers. The hearing levels of both groups were normal. The data was collected as a part of the project *Genetic Study of High Functioning Autism and Asperger's Syndrome in Finland*. Study was carried out with the agreement of the Ethical Committee of Oulu University Hospital and written informed consent was obtained from parents.

The data of this study were originally collected in order to study auditory perception of children with AS using event-related potentials (ERPs) [16-18]. It was found that children with AS had deficient auditory sensory processing and abnormal auditory brain reactions to prosodic features of speech. In this study we retrospectively analyzed the data of the test where auditory ERPs were used. In addition the participants were watching silent cartoon. We hypothesized that this kind of test protocol with an audiovisual stimuli may have been stressful for the children with AS.

B. Data Acquisition

EEG and one-channel ECG were recorded using the NeuroScan Synamps Amplifier and the NeuroScan 4.0 software (Neuro Soft Inc., Sterling,VA). EEG signals were recorded using Ag/AgCl electrodes placed according to the international 10–20 system. The sampling rate was 1000 Hz and the signals were online band pass filtered at 0.05–70 Hz.

The auditory stimuli were spoken by a female voice and presented using headphones. Two one-word utterances, Anna! (Give [it to me]! in English) which were in imperative mood representing two opposite emotional states, tender and angry, were used in the test. The length of both stimuli was 740 ms, and the inter stimulus interval was 700ms. The sound level was about 55 dB. The stimulus block, length of eight minutes, contained 400 words from which 15% were angry while the rest of the stimuli were pronounced with a tender voice. Fig. 1 presents the positions of angry words plotted on an example case of a simultaneous HRV signal. Children were instructed to ignore the sound stimuli and they were watching a silent cartoon during the data acquisition. In addition, baseline measurements were performed where subjects were asked to sit down quietly, eyes closed for 40 seconds.

C. Heart Rate Variability

R-peaks were detected and consecutive RR-intervals were defined. Series of RR-intervals were interpolated at 4Hz and linear trends were removed. Spectra were calculated using autoregressive modeling (Burg's method) with model order 16, which is generally used when analyzing RR-intervals [19]. Very low frequency (VLF 0.003-0.04Hz), LF and HF bands were integrated to obtain powers in the bands. Normalized LF and HF (LFnu and HFnu) powers were calculated by dividing LF and HF separetely with sum of LF and HF powers. In addition, LF/HF ratio and mean heart rates were calculated.

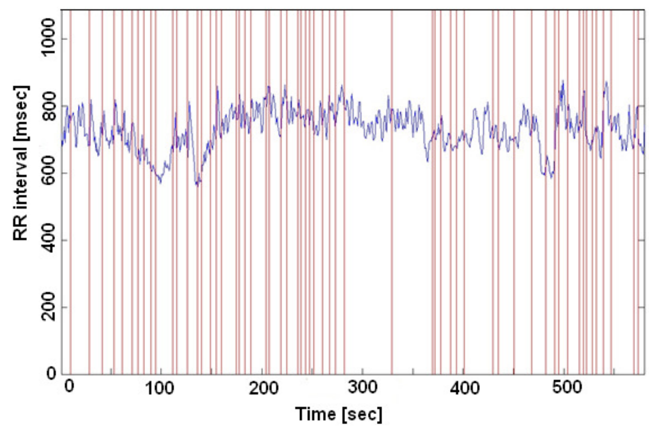

Fig. 1. Angry stimuli positions (red) during the test block and an example of a simultaneous HRV signal (blue curve).

HRV analysis was performed in one minute windows which were overlapping for two seconds through the whole test data to obtain the time evolutions during the stimulus block. Also, the values from all the windows were averaged within groups. Baseline test was analyzed for HRV indices as a one segment.

D. Frontal EEG Asymmetry and Brain Load Indices

F3, F4, Fz, Cz and Pz channels were selected for the analysis. First, linear trends and means were removed from the data. Then theta (4-7Hz), alpha (8-13Hz) and beta (14-30Hz) bands were discriminated using Chebyshev band pass filtering. Spectra were estimated using Welch's method (Hamming window, 2048 window length and 50% overlap) and powers, i.e. theta, alpha and beta activities were obtained by integrating the spectra.

Two indices that have been suggested to evaluate the stress or brain load were selected to apply in this study. Frontal EEG asymmetry index is defined as an alpha activity change between right and left prefrontal cortex (here F4 and F3 channels) [7] and it is presented in equation (1).

$$Asymmetry\ index = \ln[alpha(F4)] - \ln[alpha(F3)] \quad (1)$$

We also tested a modified asymmetry index that was calculated so that alpha(F4) and alpha(F3) activities during the test were normalized by dividing with the corresponding mean activities within the groups during the baseline measurements.

Brain load index defines the overall acute external or cumulative internal load and it is calculated from frontal and parietal lobes in a following way [9]:

$$Brain\ load\ index = \frac{theta\ (Fz)}{alpha\ (Pz)} \quad (2)$$

The EEG indices during the test were analyzed similarly in one minute overlapping windows, than in HRV analysis, through the whole data. Baseline was analyzed in four seconds windows.

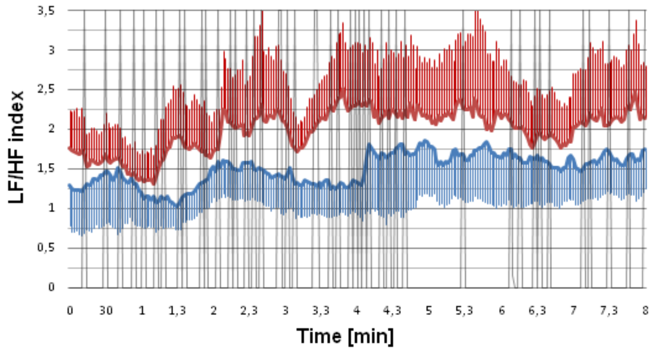

Fig.2. Time evolution of LF/HF index (red = Children with AS, blue = Controls). Determined using 1min windows and 2s stepping size.

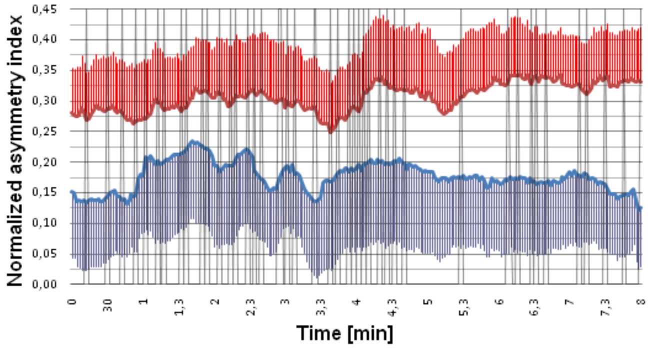

Fig.3. Time evolution of Normalized asymmetry (red = Children with AS, blue = Controls). Determined using 1min windows and 2s stepping size.

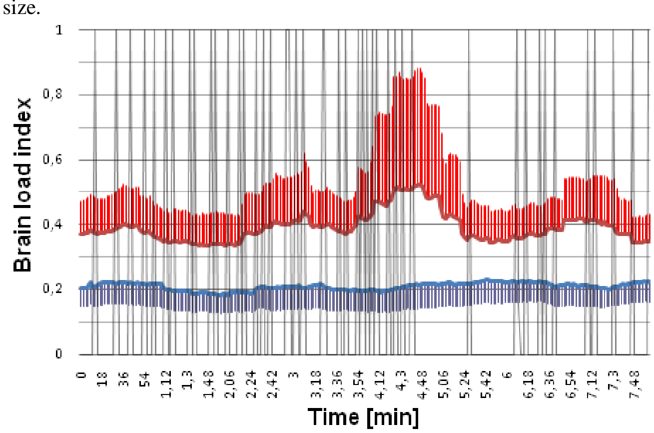

Fig.4. Time evolution of Brain load index (red = Children with AS, blue = Controls). Determined using 1min windows and 2s stepping size.

E. Statistics

All the statistics were calculated using SPSS® software (SPSS Inc, USA). Differences between and within the groups were calculated using non-parametric Mann-Whitney test and values $p < 0.05$ were considered statistically significant.

III. Results

The HRV and EEG based indices during the baseline for controls and children with AS are presented in Table I. Statistical difference between the groups were not found. Asymmetry index is nearly zero indicating that the activity in prefrontal cortex is similar in both sides. Brain load index is low for both groups.

The averaged HRV and EEG indices during the test segment in controls and children with AS are presented in Table II. Statistical difference between the groups was found from normalized asymmetry index and brain load index ($p < 0.05$). However, statistical difference between the groups were not found from HRV indices even though a slight difference is seen in LF powers and LF/HF ratio ($p = 0.21$ and $p = 0.09$, respectively).

When comparing the results from stress related test with baseline within the groups the asymmetry index and brain load index increased significantly during the test ($p < 0.05$) in controls. In children with AS parallel changes in EEG based indices were found ($p < 0.05$). In addition, the normalized LF increased, normalized HF decreased and LF/HF ratio and heart rate increased during the test ($p < 0.05$).

The time evolution of LF/HF ratio during the stress related test is presented in Fig.2. The LF/HF ratio is higher and fluctuates more in children with AS. Fig. 3 presents the time evolution of normalized asymmetry index. Normalized asymmetry is higher in children with AS during the stress related test ($p < 0.05$). Fig. 4 presents the time evolution of brain load index during the stress related test. Mean of brain load index is higher in both groups compared to baseline and significant difference between groups exists ($p < 0.05$).

Table I
Spectral indices during baseline

Parameter	Controls	Children with AS
LF power [ms^2]	1480 ± 1850	2243 ± 3230
LFnu [%]	48 ± 17	46 ± 19
HFpower [ms^2]	1693 ± 1844	3127 ± 3911
HFnu [%]	52 ± 17	54 ± 19
LFpower/HFpower	1.13 ± 0.71	1.09 ± 0.79
HR [beat/sec]	75 ± 11	72 ± 9
Asymmetry index	0.00 ± 0.08	-0.01 ± 0.08
Brain load index	0.09 ± 0.02	0.12 ± 0.03

Table II
Spectral indices during stress related test

Parameter	Controls	Children with AS
LF power [ms^2]	2063 ± 1622	2482 ± 1551
LFnu [%]	55 ± 13	61 ± 13 †
HFpower [ms^2]	1712 ± 1322	1718 ± 1376
HFnu [%]	45 ± 13	39 ± 13 †
LFpower/HFpower	1.47 ± 0.91	1.99 ± 0.99 †
HR [beat/sec]	75 ± 11	86 ± 13 †
Asymmetry index	0.22 ± 0.25	0.22 ± 0.17 †
Normalized Asymmetry index	0.18 ± 0.25†	0.31± 0.17 ** †
Brain load index	0.21 ± 0.14 †	0.41 ± 0.27 ** †

**Statistical difference between groups ($p < 0,05$)
† Statistical difference compared to baseline (Table I) within the group ($p < 0.05$)

IV. Discussion

The stress related test data was acquired using audiovisual stimuli and analyzed for children with AS and age-matched controls using HRV and EEG based stress indicators.

During the baseline there were no significant differences between the groups. The LF/HF ratio was about one, asymmetry index was about zero and brain load index was low for both groups.

Heart rate increased in the stress related test in children with AS (75 beat/s vs. 86 beat/s) compared to baseline but not in control group. Normalized LF (LFnu) increased and normalized HF (HFnu) decreased during the test in children with AS. LF/HF ratio, also called as sympathovagal balance describes the reciprocal function of autonomic nervous system. LF/HF ratio increased significantly during the test compared to baseline in the children with AS. These changes in HRV based indices within the groups are in accordance with previously published HRV observations in stress tests [3] and can also be interpreted as increased sympathetic activity. As change in HRV indices was not significant in controls it can be assumed that children with AS experience the test more stressful than control group.

During the stress related test both asymmetry index and brain load index showed an elevated activity level of the central nervous system for both groups ($p < 0.05$) compared to baseline. According to literature, negative emotions and stress may increase the alpha activity in right prefrontal cortex [6,7]. Thus it can be stated that the test with audiovisual stimuli is experienced as stressful by both groups. We noticed that normalizing asymmetry increased the asymmetry for the children with AS.

Recently published brain load index is based on the theory that the increased cognitive brain load increases frontal theta activity and decreases parietal alpha activity [8]. In this study the brain load index was low for both groups during the baseline as it should be while subjects are resting eyes closed and it can be supposed that they are not experiencing stress. The brain load index increases significantly for both groups during the stress related test compared to baseline. The brain load index is significantly larger and fluctuated more for children with AS. The time evolution of the brain load index shows that when angry-sounding auditive stimuli are given rapidly (Fig 4.) the brain load index increases while the time evolution for control groups is quite constant. This can be interpreted that children with AS react more to auditive stimuli than control group.

Retrospectively analyzed measurements were originally designed for ERP study. The stress stimulus of this study was consisted of two stimuli, an auditive and a visual. The test situation itself may be stressful. Thus, we can only analyze the overall stress level of subjects and we are not able to discriminate the stress response sources.

The usability of brain load index as an indicator of stress can be questioned since it was originally designed to illustrate both acute external and cumulative internal brain load [9]. Thus brain load index is not necessarily directly linked to amount of stress that a person is experiencing. However, the concept of stress is somewhat unambiguous and brain load index revealed the different reaction in groups during the audio-visual test.

In conclusion, HRV based indices revealed increased sympathetic activity during the test in children with AS. EEG based indices increased more during the test compared to baseline in children with AS. Thus the children with AS seems to have a greater reaction to stressful situation.

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